Sodium ASCORBATE

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Investigations on the Toxic and Teratogenic effects of GRAS substance on the Developing chick Embryo.[2]

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GRM 117

Investigations on the Toxic and Teratogenic Effects of GRAS Substances on the Developing Chick Embryo.[1]

Sodium Ascorbate

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[1]Report of investigations conducted under Contract No. 72-343 with the Food and Drug Administration, PHS, DHEW.

General Protocol:

Ten test substances were supplied by the Food and Drug Administration for testing in the chick embryo. Details on the nature and source of these substances is shown in Table i. All substances were stored at room temperature in the dark until they were used, except that the propyl gallate and phosphated mono- and di-glycerides were kept under refrigeration. Most of the substances were dissolved in a suitable solvent or suspended in a suitable liquid for injection into fertile eggs. In one instance the substance was injected directly without a solvent or carrier. Specific information about solvents, solubility of the substances and problems peculiar to individual substances will be given under specific protocol for each substance tested.

Fertile eggs used in these investigations were from a specific pathogen free flock of Dekalb 161 egg production type chickens fed a breeder ration free of antibiotics or other drugs. Eggs were stored at 55° F and a relative humidity of 80 percent for 0 to 5 days prior to use. Eggs were allowed to reach room temperature, placed on plastic flats and subjected to ultraviolet irradiation for 30 minutes. The top of each egg was cleansed by a cotton swab saturated with 70 percent ethanol, a small hole was drilled over the air cell through the shell and the test substance was injected with the aid of a 0.25 ml. tuberculin syringe fitted with a suitable needle. All equipment and glassware used to handle the test substances or their solutions or suspensions were sterilized by auto claving and every attempt was made to avoid microbiological contamination of the eggs. Following injection the hole in each egg was sealed by a drop of flexible collodion and the eggs were set in or returned to the incubators. Jamesway Model 252 Incubator-Hatchers were used and maintained at 100° F dry bulb temperature and 86° F wet bulb temperature during the first 18 days of incubation. Eggs were turned automatically each 4 hours. Eggs were candled periodically to remove dead embryos and all embryos were examined for stage of development and obvious defects. After 18 days of incubation viable embryos were transferred to hatching baskets and hatching temperature was reduced to 98.5° F dry bulb reading and humidity was increased to a 90° F wet bulb reading. Upon hatching (22nd day) chicks were examined for abnormalities and samples were cleared and alizarin stained to examine them for skeletal defects. Other embryos (50 for each substance studied) were sacrificed and samples of liver, muscle, bursa, brain, eye, spleen, heart, pancreas, lung and kidney were taken and fixed in formalin. Later tissues were embeded in paraffin, cut, stained and mounted for histopathological examination. Each sample was done in duplicate and hence a total of 10,000 tissues were examined for lesions.

Preliminary range finding experiments were conducted to find the doses of the test substances that could be used in constructing dose response curves for toxicity as measured by embryonic mortality. In two cases, the test substance was non-toxic in the largest dose that could be accommodated by injection. Specific dose response experiments using 100 or more eggs per dose and 5 or more doses of the test substance were conducted at a minimum of 3 time intervals to obtain the toxicity data reported. Solvent or sham injected controls and untreated control groups of eggs were used with each experiment. In some cases, extra trials were conducted to provide embryos for examination at critical doses of the test substances in order to further evaluate teratogenic response and obtain additional data on the nature of embryonic defects.

Data obtained from the experiments (except that from the range finding studies) was transferred to data sheets provided (FDH form 2572, 2572a and 2572b) and submitted to FDA for statistical analysis. Nine types of data summaries including 2 statistical treatments of the data were provided by FDA on the data submitted. The results presented and interpretations made are largely based on these data summaries.

Table i

FDA Project Test Substances

Test Substance and Identification		Compound No.
1. Lactose, Edible Formost Dairies, Inc. Appleton, Wisc.		000063423
2. Propyl Gallate Lot 337		000121799
3. Sodium Ascorbate, U.S.P. FCC Lot No. 965102 Hoffmann-LaRoche Inc., Nutley, N. J. FDA 3167 73(C)		000134032
4. Sodium Erythorbate F.C.C. Lot No. 834072 FDA 3167 73(C) Hoffmann-LaRoche, Nutley, N. J.		977052064
5. Oil Nutmeg NF, East Indian Fritzsche Dodge & Olcott, Inc. 71-28 New York, N. Y.		MX 8008455
6. Zinc Sulfate - Rayon Lot # 2132R1 Virginia Chemicals, Inc. Portsmouth, Va.	Anhyd. Monohyd.	007733020 007446197
7. Stannous Chloride, AR $2H_2O$ Mallinckrodt Chemical Works St. Louis, Mo.		007772998
8. Talc USP #141, Whittaker, Clark and Daniels, Inc.		010101390
9. Carob Bean Gum FDA 71-14		PM 9000402
10. Phosphated Mono- and Di-Glycerides Lot No. 126 Witco Chemical Organics Division New York, N. Y. EMCOL D70-30C		977051323

General Discussion and Comparisons:

A comparison of the relative toxicity of the ten compounds tested is shown in Table ii. When toxicity is evaluated by the air cell route of injection at 96 hrs. of incubation, which was the most sensitive for most of the substances tested, it may be seen that the test substances can be divided into 3 categories of toxicity. Substances highly toxic are zinc sulfate, propyl gallate and carob bean gum. Moderate toxicity was encountered with sodium ascorbate, sodium erythorbate, oil of nutmeg and stannous chloride. Those substances of low toxicity were lactose, talc and phosphated mono- and di-glyceride.

Most of the substances tested produced general embryo toxic response as ascites and/or edema except for lactose and talc at the doses tested. Some specific structural defects were noted and seemed to be related to certain substances as shown in Table ii.

Table ii

Comparison of Ten Substances Tested
for Toxicity and Teratology

Substance Tested	LC_{50} via air cell at 96 hrs.	Specific Abnormalities Noted
Lactose	very large	none
Propyl Gallate	13 mgs./kg.	Ascites, edema, celosomia.
Sodium Ascorbate	100 mgs./kg.	Ascites, edema, celosomia, liver histopathology, head defects.
Sodium Erythorbate	84 mgs./kg.	Ascites, liver histopathology.
Oil of Nutmeg	240 mgs./kg.	Ascites, edema, celosomia, dwarfism.
Zinc Sulfate	4 mgs./kg.	Ascites, edema, celosomia, dwarfism.
Stannous Chloride	120 mgs./kg.	Ascites, edema, celosomia.
Talc	>200 mgs./kg.	none
Carob Bean Gum	23 mgs./kg.	Anophthalmia, phocomelia, micromelia, torticollis, celosomia.
Phosphated Mono- and Di-Glycerides	>3000 mgs./kg.	Ascites, anophthalmia, brachygnathia.

III. SODIUM ASCORBATE

Specific Protocol:

Sodium ascorbate is highly water soluble and hence solutions were made using this solvent. The pH of freshly prepared ascorbate solutions was adjusted to 6.0 and the solutions were filter sterilized to prevent oxidation of the compound to dehydroascorbic acid. The solutions were used within one hour after preparation. Five doses of sodium ascorbate were tested at both 0 and 96 hrs. of incubation and via both air cell and yolk routes of administration.

Results:

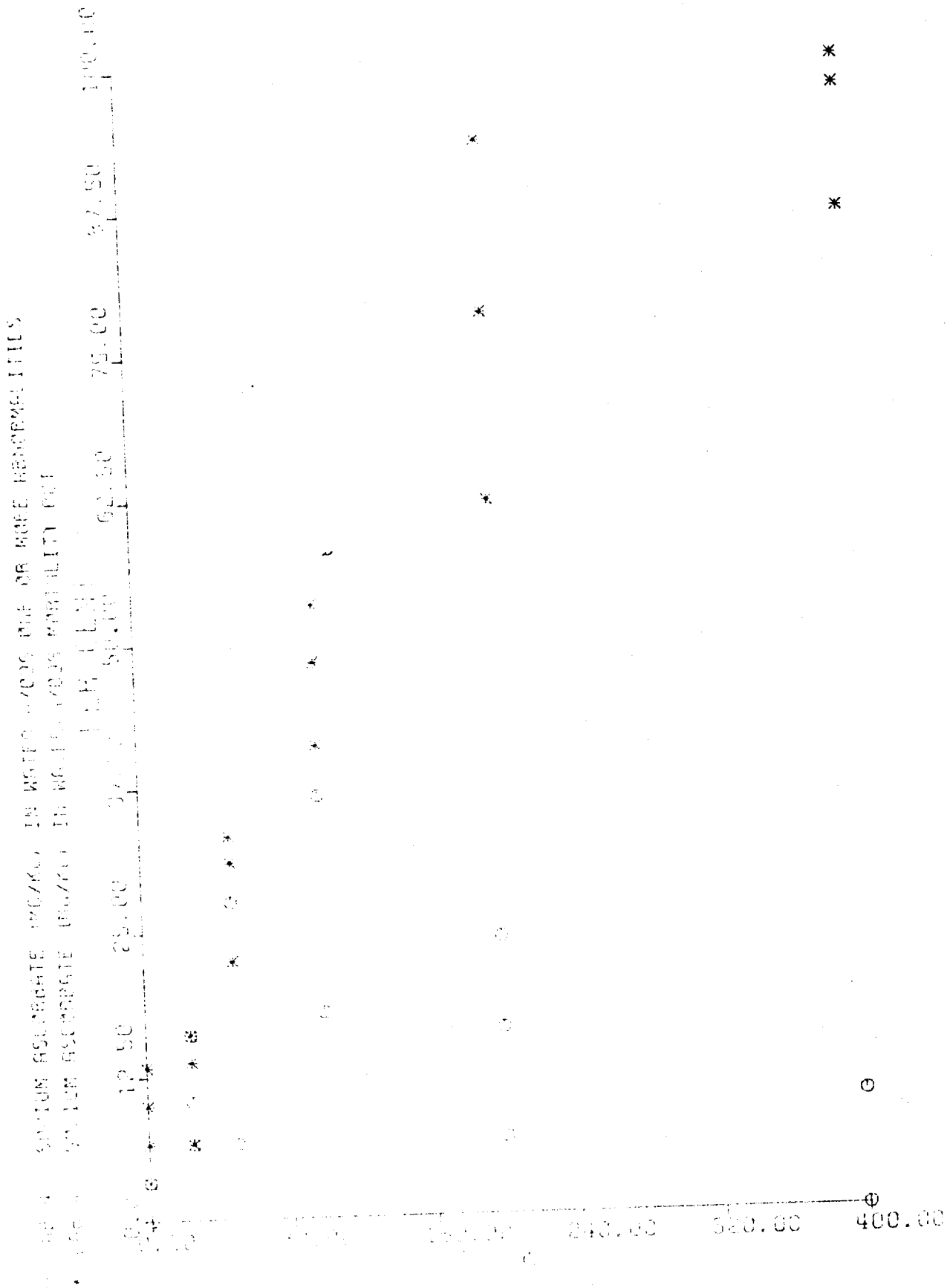
The data for sodium ascorbate is presented in Tables 9-12. Significant increases in percent mortality were observed at some of the larger doses of sodium ascorbate under all conditions of compound administration. Toxicity as measured by percent mortality was greatest at 96 hrs. via the air cell and lowest at 96 hrs. via the yolk. In one case (yolk administration at 0 hr.) mortality appeared to have been reduced by the lowest level of ascorbate when compared to the solvent controls. This result appears to be a chance event because yolk administration at 0 hr. usually leads to considerable mortality as noted previously.

Percent abnormal chicks hatched was significantly increased by higher levels of ascorbate under all conditions except that of the 20 mg./egg dose via the air cell at 96 hrs. where very high mortality was observed. At the highest level of administration significantly more H-S-V-L abnormalities were observed when the compound was given at 0 hr. via the air cell and at 96 hrs. via the yolk. The regression of dose on abnormal chicks or on 2 or more abnormalities per chicks was significant.

Among specific embryonic defects, ascites, general edema and celosomia were observed most frequently. In the area of histopathology more fatty livers showing dilatation, necrosis or metamorphosis were noted. All these conditions appeared to be more frequent at high levels of ascorbate administration. The increase in H-S-V-L abnormalities was due not only to more frequent appearance of celosomia but also due to increase in abnormalities of the head such as anophthalmia, brachygnathia and buphthalmia.

Discussion:

Sodium ascorbate clearly produces an embryo toxic response that is closely related to the dose administered. This is evidenced by a high level of embryonic mortality at larger doses and an increase in the percent of abnormal chicks hatched when mortality was between 10 and 80 percent. The histopathological findings, although suggesting some treatment effect, were not of statistically significance due to their low incidence. The LC50 at 96 hrs. via the air cell was about 100 mgs./kg.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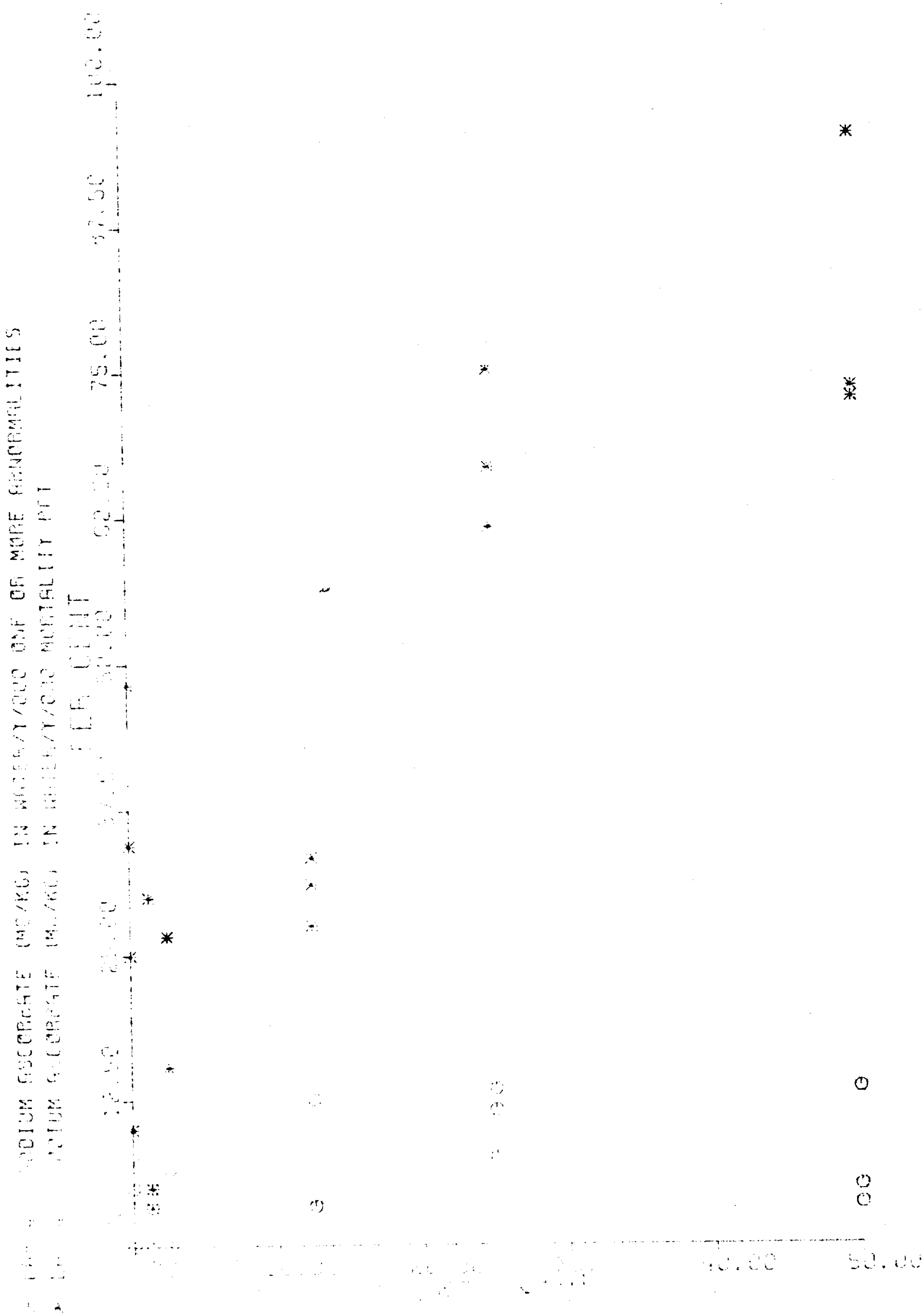
ONE OR MORE ABNORMALITIES
MORTALITY PCT
PER CENT
25.00
50.00
75.00
87.50
100.00
40.00
50.00

SODIUM CHLORIDE (MG/KG) IN WATER/SOIL ONE OR MORE RENDERABILITIES
SODIUM CHLORIDE (MG/KG) IN WATER/SOIL MORTALITY PCT
PER CENT
75.00
400.00

Table 9

DATA SUMMARY

Sodium Ascorbate in Water
via Air Cell at 0 Hr.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	636	5.18	2.51	0.47
Solvent	None	158	8.86	3.16	1.26
12.5	0.625	100	7.00	5.00	0
25.0	1.25	97	15.46	4.12	1.03
125.0	6.25	97	16.49	9.27	0
250.0	12.5	99	21.21[1]	14.14[2]	2.02
500.0	25.0	97	52.57[1]	21.64[2]	9.27[3]

[1] Difference from control group is highly significant

[2] Difference from control group response is highly significant

[3] Same as 2

[4] Regression of dose on mortality is significant

LC_{30} = 346 mgs./kg.
LC_{50} = 562 mgs./kg.
LC_{70} = 912 mgs./kg.
LC_{90} = 1836 mgs./kg.

[5] Regression of dose on abnormal chicks is significant

Table 10

DATA SUMMARY

Sodium Ascorbate in Water
via Air Cell at 96 Hrs.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	636	5.18	2.51	0.47
Solvent	None	120	8.33	2.50	0.83
25.0	1.25	90	12.22	11.11[2a]	4.44[3]
50.0	2.5	99	28.28[1]	11.11[2a]	2.02
100.0	5.0	98	46.93[1]	24.48[2]	4.08
200.0	10.0	96	78.12[1]	15.62[2]	3.12
400.0	20.0	96	94.79[1]	3.12	2.08

[1] Difference from control group is highly significant

[2] Difference from control group response is highly significant

[2a] Difference from control group response is significant

[3] NS

[4] Regression of dose on mortality is highly significant

LC_{30} = 63 mg/kg
LC_{50} = 101 mg/kg
LC_{70} = 165 mg/kg
LC_{90} = 332 mg/kg

[5] Slope is negative

Table 11

DATA SUMMARY

Sodium Ascorbate in Water
via Yolk at 0 Hr.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	636	5.18	2.15	0.47
Solvent	None	128	28.90	3.90	1.56
12.5	0.625	100	12.00[1a]	3.00	2.00
25.0	1.25	99	22.22	3.03	1.01
125.0	6.25	99	30.30	10.10	4.04[3]
250.0	12.50	96	68.75[1]	10.41[2a]	3.12
500.0	25.00	99	81.81[1]	16.16[2]	3.03

[1a] Difference from control group is significant

[1] Difference from control group is highly significant

[2] Difference from control group response is highly significant

[2a] Difference from control group response is significant

[3] NS

[4] Regression of dose on mortality is significant

LC_{30} = 196 mgs./kg.
LC_{50} = 294 mgs./kg.
LC_{70} = 441 mgs./kg.
LC_{90} = 790 mgs./kg.

[5] NS - F (Cal) $<$ F (.05)

Table 12

DATA SUMMARY

Sodium Ascorbate in Water
via Yolk at 96 Hrs.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5,5a]	Percent H-S-V-L Abnormalities
Control	None	636	5.18	2.51	0.47
Solvent	None	159	13.83	3.77	0
25.0	0.625	94	8.51	3.19	1.06
50.0	1.25	94	10.63	3.19	1.06
100.0	6.25	95	11.57	10.52[2a]	3.15
200.0	12.5	93	13.97	16.12[2]	1.07
400.0	25.0	90	25.55[1]	24.44[2]	4.44[3]

1 Difference from control group is significant

2 Difference from control group response is highly significant

2a Difference from control group response is significant

3 Same as 2a

4 NS

5 Regression of dose on abnormal chicks is significant

5a Regression of dose on 2 or more abnormalities per chick is significant.